

Open Literature Review Summary

Chemical Name(s): Clothianidin

PC Code: 044309 (Clothianidin)

MRID:

ECOTOX Record Number and Citation:

Rundlöf, M., G. K.S. Andersson, R. Bommarco, I. Fries, V. Hederström, L. Hebertsson, O. Jonsson, B. K. Klatt, T.R. Pedersen, J. Yourstone and H.G. Smith. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature*. Vol. 521. doi:10.1038/nature14420

Purpose of Review (Note: DP Barcode required for Quantitative studies): Registration Review

Study Type: Tier III Field level monitoring study on honey bees, bumble bees and solitary nesting bees

Date of Review: 04/21/16

Description of Use in Document (QUAL, QUAN, INV): Qualitative

Summary of Study Findings:

Planted oilseed rape seeds coated with clothianidin and β -cyfluthrin (Elado, Bayer, 400 g/L clothianidin, 180 g/L β -cyfluthrin per kg seed) altered wild and managed bee species under field conditions in Swedish agricultural landscapes. Eight pairs (n=16) of landscapes surrounding spring-sown rape fields for which one field in each of the eight pairs was sown with Elado (considered the insecticide-treated seeds) and a fungicide (thiram) and the other field in the pair was sown with just the fungicide (considered the control) [7.5 kg/ha for thiram-treated seeds and 7.7 kg/ha for Elado+thiram-treated seeds]. The study authors describe four major findings. First, at the insecticide-treated sites and adjacent uncultivated field borders, density (number) of bumblebees (*Bombus terrestris* L complex (multiple B. species)) and solitary bees (*Osmia bicornis* L.) [together referred to as “wild bees”] was reduced compared to control fields. Wild bee density increased with the size of the focal oilseed rape field, but was not significantly related to the proportion of agricultural land in the surrounding landscape. Flower cover (number and size) had a positive effect on wild bee density and was higher in insecticide-treated fields, but wild bee density was still reduced when flower cover was included as a covariate. Second, nesting of solitary bees was reduced at the insecticide-treated fields compared to control fields, in that 6 out of 8 females in the control fields but none of the insecticide-treated fields started to build brood cells. Thirdly, colony growth (based on colony weight) and reproduction of the bumblebee was affected in treated fields compared to controls. The change and rate of colony

weights was reduced, as well as fewer queen and worker/male cocoons were produced in treated fields compared to controls. Fourth, the insecticide seed treatment had no significant impact on honeybee (*Apis mellifera*) colony strength (based on number of adult bees). For the solitary bees, 35% was oilseed rape pollen from the collected pollen at the control sites (since bees at treated fields did not display nesting activity, no pollen was collected). For bumblebees, the amount of oilseed rape pollen collected from the control and treated fields was similar (~80%). For honeybees, similar rates of oilseed pollen collected was observed between control and treated fields (~58%). Clothianidin residues were detected in the nectar and pollen collected by bees and β -cyfluthrin was not. Honeybee pollen and nectar contained residue concentrations of clothianidin of 6.6-23 [mean 13.9] ng/g and 6.7-16 ng/mL [mean 10.3], respectively. Bumblebee nectar concentrations of clothianidin were 1.4-14 ng/mL [mean 5.4]. Clothianidin residues in field boarder plants (within 2 weeks of sowing) ranged from 0-6.5 ng/g [mean 1.2 and 1.0].

Materials and Methods

Study Design

Eight pairs of fields located in southern Sweden in 2013, paired based on land use, were used in the study [original study design was to use 20 fields, but some fields were excluded based on land cover, potentially previous neonicotinoid use]. The 16 fields were spatially separated (>4 km), spring-sown with oilseed rape (Majong cultivar) fields (mean \pm s.e.m. field size 8.9 \pm 1.4 ha, range 4–27 ha). Land surrounding the fields included agricultural land (ranging from 6-88%) along with other land types including grassland, forest and urban land (see Extended data table 1 in paper). One field in each of the eight pairs was randomly chosen to be sown with oilseed rape seeds treated with Elado (Bayer, 400 g/L clothianidin, 180 g/L β -cyfluthrin per kg seed; considered the insecticide-treated seeds; 0.06 lb a.i./A based on EPA information (see reviewer's calculation section); 0.06 mg clothianidin a.i./seed based on the verified use information for Canada (seeding rate of 181,000 seeds/kg seed)) and a fungicide (thiram) and the other field in the pair was sown with just the fungicide (considered the control) [7.5 kg/ha for thiram-treated seeds and 7.7 kg/ha for Elado+thiram-treated seeds]. The sowing rate was 150 plants/m², and the fields were planted from April 6 to May 18, 2013 (in two pairs, the treated fields were planted considerably earlier (~2wks) than the control fields). Fields were treated with other plant protection products (Avaunt: indoxacarb, Mavrik: t-fluvalinate, Plenum: pymetrozine, and Steward: indoxacarb) including one control field with another neonicotinoid chemical, thiacloprid.

Wild bees and/or flower cover in the oilseed rape fields and adjacent field borders were monitored on three occasions during the study (inspection dates from June 17 - July 16, 2013; see Extended Data Table 2 below). Four in field transects of 2x25 m located 2–4 m from the edge of the oilseed rape field were surveyed twice (18 June to 12 July and 27 June to 16 July). Transects of 2x300m located at the outer 1-m edge of the oilseed rape field and 1 m of the adjacent, uncultivated field border were surveyed once (17 June to 8 July). Surveys were conducted on warm days with no rain and light wind. All flower visiting and flying solitary bees

and bumblebees within the transects were noted and taxonomy determined to extent possible. Bumblebees belonging to *Bombus terrestris* complex could not be separated and therefore were grouped. Flower cover was calculated based on number and size of flowers within transects.

Extended Data Table 2 | Phenology (date, BBCH³³ and flower cover) in the oilseed rape fields and delivery, placement and survey* of bees

Pair	Seed treatment [†]	Sowing date	Date placement <i>Osmia bicornis</i> (oilseed rape growth stage (BBCH))	Date placement <i>Bombus terrestris</i> (oilseed rape growth stage (BBCH))	Date placement <i>Bombus terrestris</i> (oilseed rape growth stage (BBCH))	Date placement <i>Apis mellifera</i> (oilseed rape growth stage (BBCH))	Date wild bee survey border (oilseed rape growth stage (BBCH))	Date wild bee survey field 1 (% flower cover)	Date wild bee survey field 2 (% flower cover)
			<i>Osmia bicornis</i> (oilseed rape growth stage (BBCH))	<i>Bombus terrestris</i> (oilseed rape growth stage (BBCH))	<i>Bombus terrestris</i> (oilseed rape growth stage (BBCH))	<i>Apis mellifera</i> (oilseed rape growth stage (BBCH))	<i>Apis mellifera</i> (oilseed rape growth stage (BBCH))	<i>Apis mellifera</i> (oilseed rape growth stage (BBCH))	<i>Apis mellifera</i> (oilseed rape growth stage (BBCH))
P01	contr	23 April 2013	13 June (59)	18 June (2)	20 June (65)	19 June (65)	25 June (65)	1 July (52)	3 July (43)
P01	treat	28 April 2013	13 June (57)	18 June (2)	20 June (61)	19 June (61)	25 June (63)	1 July (95)	3 July (97)
P02	contr	7-8 May 2013 [‡]	13 June (50)	20 June (3)	26 June (63)	25 June (63)	6 July (65)	28 June (58)	9 July (60)
P02	treat	21 April 2013 [‡]	13 June (61)	18 June (2)	18 June (63)	18 June (63)	20 June (63)	19 June (90)	27 June (49)
P03	contr	18 May 2013	24 June (52)	25 June (4)	28 June (60)	2 July (61)	8 July (63)	12 July (33)	16 July (46)
P03	treat	11 May 2013	24 June (57)	25 June (4)	28 June (61)	2 July (63)	8 July (65)	8 July (53)	12 July (64)
P04	contr	6 May 2013 [‡]	13 June (50)	20 June (3)	26 June (65)	25 June (65)	4 July (65)	7 July (58)	9 July (61)
P04	treat	21 April 2013 [‡]	13 June (61)	18 June (2)	18 June (63)	18 June (63)	20 June (65)	19 June (89)	1 July (37)
P05	contr	29 April 2013	15 June (57)	18 June (2)	20 June (63)	20 June (63)	24 June (65)	24 June (21)	4 July (39)
P05	treat	25 April 2013	15 June (61)	18 June (2)	18 June (63)	18 June (63)	24 June (65)	24 June (57)	4 July (100)
P06	contr	1 May 2013	13 June (57)	18 June (2)	19 June (63)	19 June (63)	28 June (65)	2 July (74)	5 July (94)
P06	treat	25-26 April 2013	13 June (53)	18 June (2)	19 June (63)	19 June (63)	28 June (65)	5 July (89)	9 July (81)
P07	contr	4 May 2013	15 June (55)	20 June (3)	24 June (63)	24 June (63)	1 July (65)	7 July (26)	11 July (33)
P07	treat	2 May 2013	15 June (57)	20 June (3)	24 June (64)	24 June (64)	1 July (65)	7 July (87)	11 July (39)
P08	contr	6 April 2013	10 June (61)	11 June (1)	14 June (65)	14 June (65)	17 June (65)	18 June (43)	28 June (5)
P08	treat	16 April 2013	10 June (61)	11 June (1)	14 June (63)	14 June (63)	18 June (65)	18 June (14)	28 June (72)

*Shaded numbers are surveys selected for analysis of wild bee density data collected at the same time (that is, within subsequent days) within the field pairs.

[†]contr, control; treat, insecticide seed coating.

[‡]Highly asynchronous phenology of the fields within the pair.

Osmia bicornis

Solitary bee (*Osmia bicornis*) nests (n=3) were placed at each field approximately a week before the latest field within a pair was estimated to start flowering (stage 55-63 BBCH scale) between June 10 and 24, 2013. A total of 48 nests were tested. Emergence from the cocoons was artificially delayed by storing cocoons at 2-5°C to match the phenology of the oilseed rape. Each of the three trap nests contained 29 paper tubes and nine *O. bicornis* cocoons (4 females, 5 males) for a total of 27 cocoons (12 females, 15 males). The trap nests were mounted on poles in the field borders, approximately 50 m apart, facing southwards and with sheltering vegetation on the northern side. Nesting tubes were collected 36-43 days after installation, and nesting activity was determined in October 2013 by counting the number of tubes with brood cells. Proportion emerging was determined by counting the number of open cocoons. Pupa was considered dead if the cocoon was still intact 4 weeks after placement in field.

Bombus terrestris

Commercially reared bumblebee colonies (*Bombus terrestris*) (n=6) were placed at each field at the start of oilseed rape flowering (between June 14 and 28, 2013). A total of 96 hives were tested. Colonies were approximately 10 weeks old and contained one queen and approximately 50 workers and brood in all stages. Evaluation of pathogens or parasites in the colonies were not quantified prior to placement. Colonies were placed in triplets in two ventilated houses, located in a shaded part of the field borders, and did not receive any supplementary feeding after placement. The inner plastic boxes and its contents (bees, brood, and nesting material) were weighed when placed and biweekly thereafter. All colonies were terminated by freezing at the first sight of emerging new queens in any of the 12 colonies (between July 7 and August 5, or 23-38 days after placement). The two outer colonies in each triplet box were examined to estimate the number of queens and worker/male cocoons, weight of cocoons, larvae and nest structure, number of cells used for nectar and pollen storage. Separation between queen and worker/male cocoons were based on the lowest value between the peaks of the bimodal distribution of cocoon width, based on measurement of all cocoons from four of the colonies. Honeybee colonies (*Apis mellifera*) (n=6) were placed at each field on the start of oilseed rape flowering between June 14-July 2, 2013. A total of 96 hives were tested. Each colony contained estimated $3,418 \pm 123$ (mean \pm s.e.m.) adult bees per colony (with no statistical difference between treatments). Honeybee strength (number of adults per colony) was assessed before placement and again an over-wintering location after removal from test sites at the end of the flowering period on 2 - 31 July (18-33 days after placement; reviewer calculated). Colonies were equalized to include two full honeycombs with bees, two combs with mainly sealed brood with bees, one queen originating from the same colony as the one from which the split (newly created colony) was taken, bees from two combs shaken into the split, one drawn out empty comb and five combs with wax foundation. Prior to placement, colonies were placed in field of organic winter-sown oilseed rape (60 ha field).

Pollen Samples

Pollen samples were collected from pollen traps mounted on honey bee colonies, bumble bees foraging in the field, and solitary bee brood cells. At least 25 mL of pollen was collected from each field. A subsample of 15.0 g of the *A. mellifera*-collected pollen was sorted into separate samples based on colour and the separate samples were weighted. One to five samples from *B. terrestris* were collected per field (2.9 ± 0.3), giving a total of 47 samples. Pollen was collected, when possible, from *O. bicornis* larval cells, resulting in 17 samples from the six control fields with nesting activity. Fifty to 500 random pollen grains were sampled and phenology was determined (from either oilseed rape or other plant species).

Neonicotinoid Residues

Vegetation, pollen and nectar were sampled and analyzed for neonicotinoid residues along with other chemicals. Along the transects used for wild bee monitoring, flower and leaves were collected within 2 days of planting (7 April – May 20) in the permanent field borders adjacent to the oilseed rape fields. Additionally, samples from the treated fields were collected 13-15 days after planting (21 April – June 3).

Pollen Samples for Neonicotinoid Residues

In each field, five *A. mellifera* with pollen loads were caught to collect pollen samples and at least five nectar foragers were caught to collect nectar from the honey stomach. At two of the control fields, no *A. mellifera* with pollen loads could be found in the oilseed rape fields. Five *B. terrestris* were caught in the flowering oilseed rape fields, brought to the laboratory and nectar was extracted from the nectar stomachs of 3–5 bees per field, except at one control field where only one bee carried nectar.

Nectar Samples for Neonicotinoid Residues

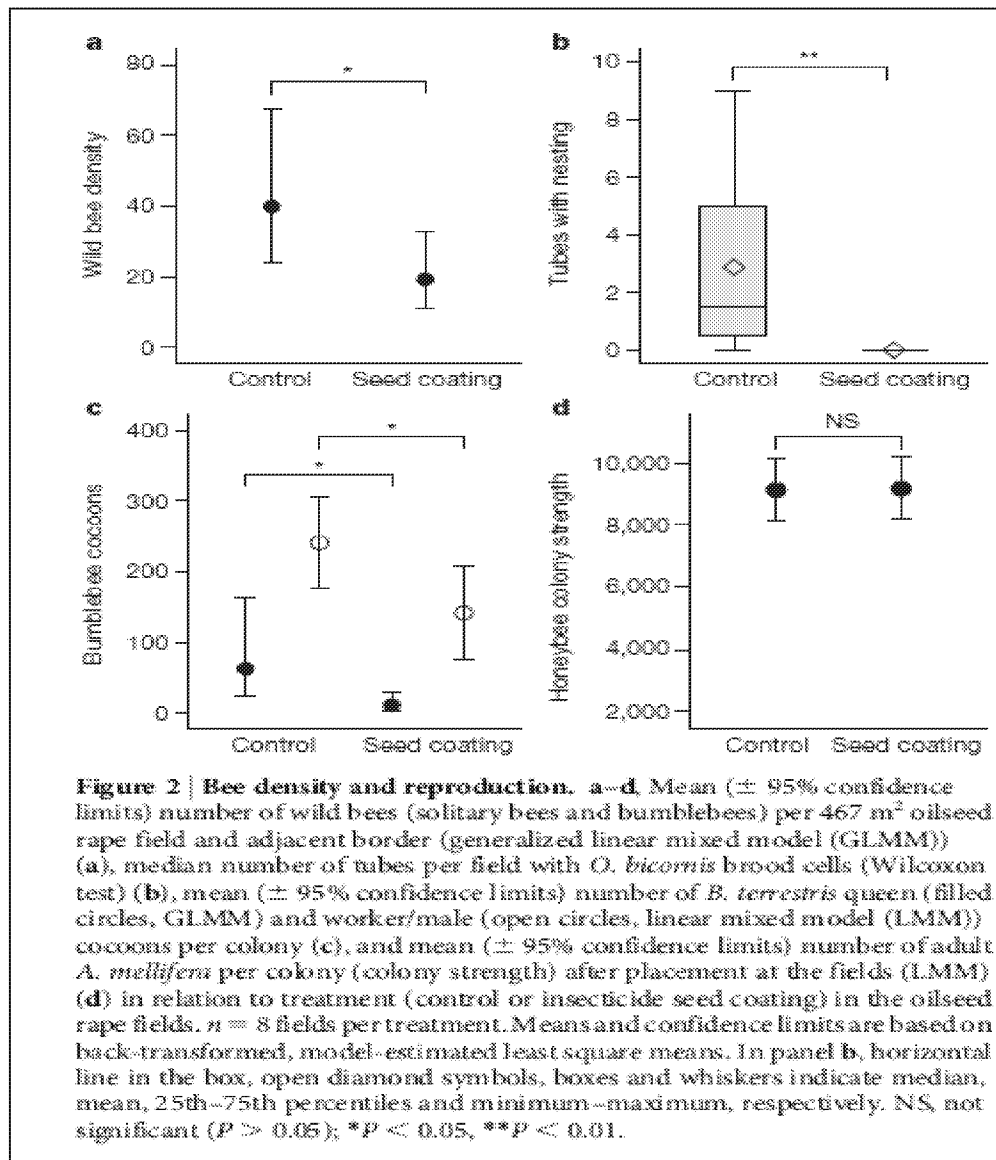
Nectar samples were quantitatively handled using the capillary microsampling technique. Neonicotinoids were quantified using liquid chromatography coupled with tandem mass spectrometry. Beta-Cyfluthrin was quantified using gas chromatography coupled with mass spectrometry. See Extended Data Table 8 for limits of detection and quantification.

Personnel monitoring bees in the oilseed rape fields, handling the solitary bee nests, weighing and examining bumble bee colonies, assessing honey bee colony strength, and collecting honey bee pollen and nectar samples were blinded to treatment. Persons collecting vegetation samples and wild bees in border transects, and collecting bumblebees for pollen and nectar samples were not blinded. All data was analyzed using SAS 9.4. Wild bee densities were compared between treatments and in relation to flower cover, size of the focal oilseed rape field and proportion of agricultural land in the surrounding landscape using a generalized linear mixed model (GLMM, SAS PROC GLIMMIX) with Poisson error distribution and log link. Pair identity, pair identity x treatment and field part nested within pair identity x treatment were included as random factors, to account for the pairing of sites and the hierarchical study design. GLMM with binomial error distribution and logit link were used to test the difference in flower cover between treatments, both for all data and for only temporally synchronous surveys. Differences in emergence of *O. bicornis* from the cocoons between treatments, sexes and their interaction were tested with a GLMM with binomial error distribution and logit link. Pair identity, pair identity x treatment and sex nested within pair identity x treatment were included as random factors. The number of *O. bicornis* nest tubes with nesting activity was compared between treatments using Wilcoxon–Mann–Whitney test. An individual growth model based on a linear mixed model (LMM, SAS PROC MIXED) was used to test the effect of treatments on the weight gain of the *B. terrestris* colonies from placement at the fields (day 50). The net weight gain was related to day, treatment, day x treatment, day x day and day x day x treatment. Random intercepts and random slopes for day and day x day were included, with the colony identity as the subject and an unstructured covariance matrix. Pair identity and pair identity x treatment were included as random factors to account for the study design. Honey bee colony strength (that is, number of adult bees per colony) was compared between treatments using a LMM. Colony strength before placement at the fields was used as a covariate and pair identity and pair identity x treatment were included as random factors. A power analysis for honey bee colony strength was conducted, and, based on the test design, the analysis would be able to detect an effect size of just under 20% with a power of 0.8.

Results

Wild bee density, bumble bee reproduction, and honey bee colony strength results are presented in Figure 2 below.

- a) Wild bee density (which includes the number of solitary and bumble bees) was significantly lower in the insecticide-treated fields compared to the control.
- b) For solitary bees (*O. bicornis*), the median number of tubes per field was significantly lower in the treated fields.
- c) For bumble bees, the mean number of queen and worker/male cocoons per colony was also significantly lower.
- d) Honey bee colony strength was not significantly different between the treated and untreated fields.



Effects on Wild Bee Density

The authors also noted that wild bee density increased in correlation with the test field size but was not correlated with the proportion of agricultural land in the surrounding landscape (Extended Data Table 4). Flower cover (which included the number and size of flowers) had a significantly positive influence on wild bee density and there was a significantly higher coverage

in the treated oilseed rape fields (Extended Data Table 5). However according to the authors, the negative impact of the seed coating on wild bee density persisted irrespective of whether or not flower cover was included as a covariate in the statistical model (Extended Data Table 4).

Effects on *O. bicornis* Nesting

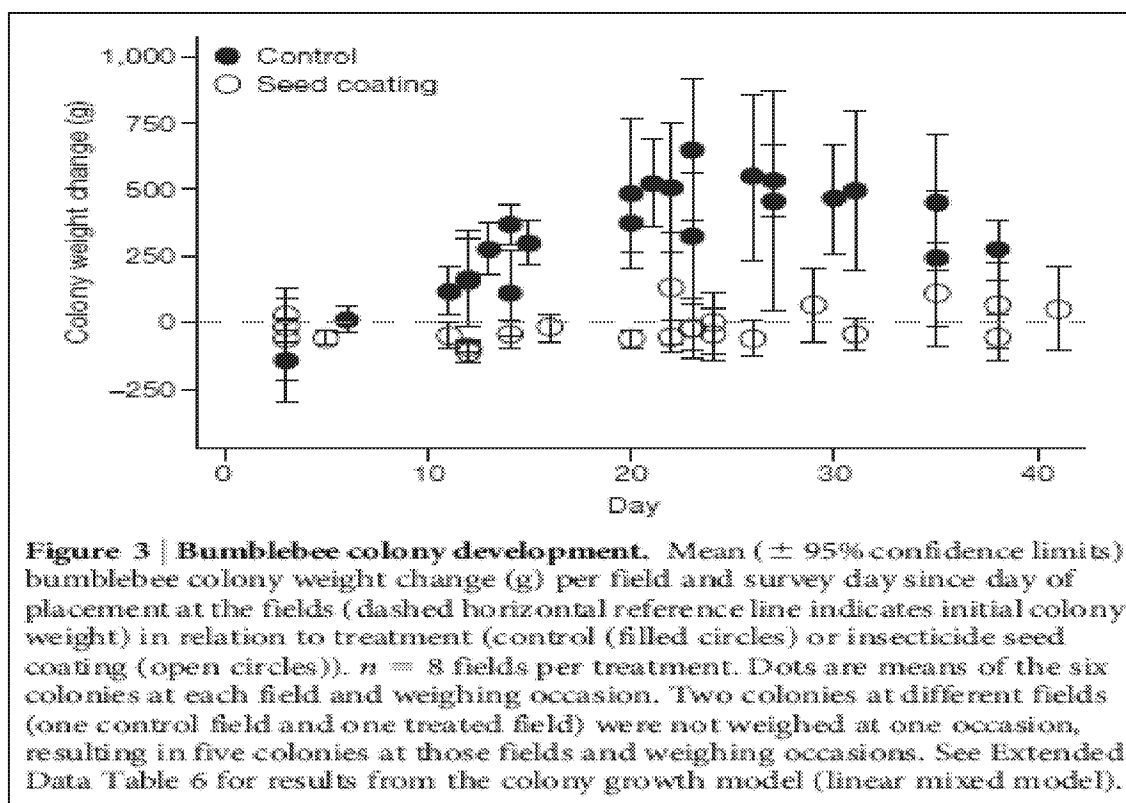
Out of the three trap nests containing 27 *O. bicornis* cocoons adjacent to each of the 16 fields before the beginning of oilseed rape flowering, females in 6/8 control fields, 0/8 in the treated fields started to build brood cells (Fig. 2b).

Effects on Colony Growth and Reproduction of *B. terrestris*

The seed-coating treatment influenced the weight change of (Extended Data Table 6 and Fig. 3); *B. terrestris* colonies placed in control fields had an initial growth stage followed by a decline while the colonies placed in the treated fields had a considerably smaller weight change (Extended Data Table 6 and Fig. 3). While the initial colony weight was the same in the two treatments (Extended Data Table 5), the rate of weight gain of colonies in the treated fields was significantly lower than that of colonies at control fields ($P < 0.001$; Extended Data Table 5). Bumblebees have an annual life cycle where only the new queens produced at the end of the season hibernate and form new colonies the following spring. At the end of this experiment, significantly fewer queen and worker/male cocoons were produced in treated fields compared to control fields (Fig. 2c and Extended Data Table 5).

Effects on Honey Bee Colony Strength

In contrast to the *B. terrestris* colonies, the *A. mellifera* colonies did not differ in strength (number of adult bees) between the treatments after placement at the oilseed rape fields (Fig. 2d). To estimate exposure the study authors assessed the transfer of clothianidin from plant to bee by first estimating the proportion of oilseed rape pollen collected by all three bee species, *O. bicornis*, *B. terrestris* and *A. mellifera* (Extended Data Table 6) and then quantifying the concentrations of clothianidin in bee-collected pollen and nectar (Table 1).



Residue Analysis

Residue analyses for clothianidin (Table 1) and other chemicals (Extended Data Table 8) are presented below. Amount of oilseed rape pollen collected is in Extended Data Table 5.

For *O. bicornis*, the authors found oilseed rape pollen in 9/17 examined cells, accounting for $35.1 \pm 17.0\%$ of the collected pollen (Extended Data Table 5). Because there was no nesting activity in the treated fields, the authors could not assess pollen collection there. For *B. terrestris*, the authors found that in the 47 pollen samples collected from bees foraging in the oilseed rape fields, overall for treated and untreated fields $80.1 \pm 5.0\%$ of the pollen was from oilseed rape, with 74.9% and 88.1% collected from the treated and control fields respectively (Extended Data Table 5). For *A. mellifera* the pollen extracted from pollen traps mounted on the hives contained on average $57.8 \pm 5.0\%$ oilseed rape pollen, with 63.1% and 52.6% collected from the treated and control fields respectively (Extended Data Table 5).

- No β -cyfluthrin was detected (Extended Data Table 8), but both pollen (13.9 ng/g) and nectar (10.3 ng/g) collected by *A. mellifera* and nectar (5.4 ng/g) collected by *B. terrestris* foraging in the oilseed rape fields contained concentrations of clothianidin that were substantially higher in the treated fields than in control fields (0, 0.1, and 0 ng/g respectively; Table 1).
- There were higher clothianidin concentrations in plants collected in field borders adjacent to treated fields ($1.2 \text{ ng/g} \leq 2$ days after sowing, 1.0 ng/g 2 weeks after sowing) than adjacent to control fields ($0 \text{ ng/g} \leq 2$ days after sowing, no material collected 2 weeks after sowing; Table 1).

Table 1 | Clothianidin concentrations in bee-collected pollen (ng g^{-1}) and nectar (ng ml^{-1}), and field border plants (ng g^{-1}), and tests of differences between treatments (control or insecticide-coated seeds)

	Control		Insecticide seed coating		Wilcoxon test for difference between treatments ($n = 8^*$)	
	Range	Mean \pm s.e.m.	Range	Mean \pm s.e.m.	Z	P
Honeybee pollen	0	0	6.6–23	13.9 ± 1.8	-3.16	0.0016
Honeybee nectar	0–0.61	0.1 ± 0.1	6.7–16	10.3 ± 1.3	-3.40	<0.001
Bumblebee nectar	0	0	1.4–14	5.4 ± 1.4	-3.53	<0.001
Field border plants (≤ 2 days after sowing)	0	0	0–5.9	1.2 ± 0.8	-2.90	0.0037
Field border plants (2 weeks after sowing)	No material collected		0–6.5	1.0 ± 0.8		

* $n = 6$ for pollen collected by honeybees at control fields, because no such bees with pollen could be found at two fields; and $n = 7$ for field border plants collected within 2 days of sowing in both treatments, because of lack of communication regarding the sowing date between the farmer and the investigator collecting the samples.

Extended Data Table 8 | Residues of neonicotinoids (n) and a pyrethroid (p) in bee-collected pollen and nectar from control fields and fields sown with insecticide treated seeds

	Control (<i>n</i> = 8 fields*)		Insecticide seed coating (<i>n</i> = 8 fields)		LOD [†]	LOQ [‡]
	Detected in <i>n</i> samples	Highest concentration	Detected in <i>n</i> samples	Highest concentration		
Honey bee pollen (ng/g)						
Acetamiprid (n)	1	0.34	0		0.080	0.24
Clothianidin (n)	0		8	23	0.50	1.5
Imidacloprid (n)	1	0.23 [‡]	0		0.30	0.90
Thiacloprid (n)	3	1.4 [§]	4	0.29	0.070	0.21
Thiamethoxam (n)	0		0		0.10	0.30
Beta-cyfluthrin (p)			0		1.0	
Honey bee nectar (ng/ml)						
Acetamiprid (n)	0		0		0.033	0.10
Clothianidin (n)	2	0.61	8	16	0.17	0.50
Imidacloprid (n)	3	0.35	0		0.17	0.50
Thiacloprid (n)	2	0.35 [§]	2	0.044	0.033	0.10
Thiamethoxam (n)	1	0.19	0		0.17	0.50

* $n = 6$ for pollen collected by honeybees at control fields, because no such bees with pollen could be found at two fields.

†LOD, limit of detection; LOQ, limit of quantification.

Pollen LOD and LOQ were estimated from spiking experiments of the average sample weight of 0.056 g.

Nectar LOD and LOQ were estimated for the 0.016 ml sample volume.

‡ Sample weight of 0.051 g explains reported value slightly below the estimated limit of detection, based on a 0.056 g sample weight.

§ One oilseed rape field sprayed with Biscaya (12 June 2013), where thiacloprid is the active ingredient (Extended Data Table 3).

Extended Data Table 4 | Wild bee density in oilseed rape fields and borders in relation to insecticide seed treatment and covariates

Model	Explanatory variable	Estimate	Degrees of freedom	F	P
Wild bees (all data)	Intercept	2.55			
	Treatment	0.73	1, 7	9.68	0.019
	Flower cover	1.06	1, 24	18.57	<0.001
	Field size	0.07	1, 7	7.46	0.028
	Proportion agricultural land	-1.20	1, 8	2.35	0.16
Wild bees (synchronized data*)	Intercept	2.03			
	Treatment	0.76	1, 6	6.69	0.043
	Flower cover	1.32	1, 29	26.56	<0.001
	Field size	0.08	1, 7	8.46	0.038
	Proportion agricultural land	-1.00	1, 5	2.76	0.15
Wild bees excluding <i>Bombus terrestris</i> ag. (all data)	Intercept	0.79			
	Treatment	1.14	1, 7	12.65	0.0096
	Flower cover	1.06	1, 17	8.52	0.094
	Field size	0.08	1, 6	6.63	0.045
	Proportion agricultural land	-0.33	1, 7	0.20	0.67
Wild bees excluding <i>Bombus terrestris</i> ag. (synchronized data*)	Intercept	-16.07			
	Treatment	9.16	1, 4	12.28	0.025
	Flower cover	2.17	1, 7	0.35	0.57
	Field size	1.77	1, 7	54.65	<0.001
	Proportion agricultural land	4.86	1, 7	1.07	0.34
Wild bees (excluding the field pair where Biscaya was used at the control field)	Intercept	0.93			
	Treatment	0.95	1, 3	20.20	0.023
	Flower cover	1.18	1, 15	16.29	0.0011
	Field size	0.20	1, 4	10.04	0.034
	Proportion agricultural land	-0.42	1, 8	0.12	0.74

Extended Data Table 5 | Statistical tests and mean values for bee-related variables in relation to the insecticide seed treatment in the oilseed rape fields

Dependent variable	Degrees of freedom	F	P	Control (mean \pm s.e.m.)	Insecticide seed coating (mean \pm s.e.m.)
Flower cover (%) - all data	1, 7	9.34	0.018	46.4 \pm 7.3	70.2 \pm 6.5
Flower cover (%) - synchronized data*	1, 6	6.28	0.028	41.4 \pm 9.0	70.9 \pm 6.0
Initial <i>Bombus terrestris</i> colony weight (g)	1, 7	0.99	0.35	733.2 \pm 17.8	722.7 \pm 18.6
Slope of <i>Bombus terrestris</i> colony growth	1, 7	115.80	<0.001	21.3 \pm 1.6	0.4 \pm 1.6
Slope of <i>Bombus terrestris</i> colony growth - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	143.02	<0.001	18.9 \pm 1.1	-0.5 \pm 1.1
Slope of <i>Bombus terrestris</i> colony growth - excluding the field pair where Biscaya was used at the control field	1, 6	106.41	<0.001	22.2 \pm 1.7	0.5 \pm 1.7
Number of <i>Bombus terrestris</i> queen cocoons	1, 7	7.78	0.027	70.0 \pm 12.3	20.6 \pm 8.3
Number of queen cocoons - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	3.82	0.11	59.7 \pm 15.8	22.0 \pm 9.6
Number of queen cocoons - excluding the field pair where Biscaya was used at the control field	1, 6	9.46	0.022	69.1 \pm 13.7	16.1 \pm 7.0
Number of <i>Bombus terrestris</i> worker/male cocoons	1, 7	8.09	0.025	241.0 \pm 29.8	142.0 \pm 29.8
Number of worker/male cocoons - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	6.57	0.050	206.1 \pm 26.3	115.6 \pm 20.7
Number of worker/male cocoons - excluding the field pair where Biscaya was used at the control field	1, 6	6.74	0.041	247.6 \pm 33.9	144.0 \pm 33.9
Weight of <i>Bombus terrestris</i> cocoons (g)	1, 7	14.77	0.0061	172.0 \pm 32.3	54.0 \pm 18.7
Weight of cocoons (g) - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	12.34	0.017	135.1 \pm 25.3	41.6 \pm 14.5
Weight of cocoons (g) - excluding the field pair where Biscaya was used at the control field	1, 6	9.62	0.021	201.1 \pm 32.3	69.2 \pm 32.3
Weight of <i>Bombus terrestris</i> larvae (g)	1, 7	0.15	0.71	15.5 \pm 6.0	13.6 \pm 5.7
Weight of <i>Bombus terrestris</i> nest structure (g)	1, 7	12.34	0.0098	261.0 \pm 24.7	139.4 \pm 24.7
Number of nectar cells	1, 7	2.43	0.16	59.4 \pm 23.7	23.5 \pm 10.4
Number of pollen cells	1, 7	0.60	0.46	5.5 \pm 2.1	3.6 \pm 1.4
Initial number of <i>Apis mellifera</i> per colony	1, 7	0.12	0.74	3412 \pm 192	3326 \pm 160
Proportion oilseed rape pollen from <i>Osmia bicornis</i> (%)				35.1 \pm 17.0	
Proportion oilseed rape pollen from <i>Bombus terrestris</i> (%)	1, 8	3.70	0.092	86.1 \pm 5.0	74.9 \pm 7.7
Proportion oilseed rape pollen from <i>Apis mellifera</i> (%)	1, 7	1.09	0.33	52.6 \pm 7.2	63.1 \pm 6.9

Extended Data Table 6 | Bumblebee colony growth (net weight gain) and honeybee colony strength (adult bees per hive) in relation to insecticide seed treatment

Model	Explanatory variable(s)	Estimate	Degrees of freedom	F	P
<i>B. terrestris</i> colony growth					
All fields	Intercept	-51.07			
	Treatment	-434.27	1, 18	51.41	<0.001
	Day	0.23	1, 21	144.31	<0.001
	Day × treatment	72.50	1, 21	143.00	<0.001
	Day × day	0.08	1, 19	102.52	<0.001
	Day × day × treatment	-1.40	1, 19	130.62	<0.001
Only control fields	Intercept	-533.40			
	Day	77.59	1, 31	129.10	<0.001
	Day × day	-1.44	1, 28	114.70	<0.001
Only fields with insecticide seed coating	Intercept	-36.53			
	Day	-1.61	1, 16	0.92	0.35
	Day × day	0.13	1, 14	10.78	0.0055
<i>A. mellifera</i> colony strength					
All fields	Intercept	9834.46			
	Initial colony strength	-0.19	1, 64	1.67	0.20
	Treatment	-41.51	1, 7	0.01	0.94
Excluding the two field pairs with other spring sown oilseed rape field within 1 km	Intercept	9609.95			
	Initial colony strength	-0.18	1, 45	1.33	0.26
	Treatment	199.73	1, 5	0.11	0.76
Excluding the field pair where Biscaya was used at the control field	Intercept	9715.31			
	Initial colony strength	-0.16	1, 57	0.82	0.37
	Treatment	90.68	1, 8	0.02	0.88

Reviewer's rate calculation:

The application rate was calculated in mass/acre (using two different methods) using information provided in the study report and outside data sources (seeds per pound based on information from USEPA Becker 2010 – 90,000 to 115,000 seeds per pound; percent clothianidin in Elado based on personal email communication from Bayer CropScience, June 13th [For Elado, the clothianidin percent wt/wt is 33.3% (= 400 g/l), and the beta cyfluthrin is 6.7% (= 80 g/l), based on the 1.2 g/mL density].

1. $0.025 \text{ L/kg seed} \times 400 \text{ g a.i./L} = 10 \text{ g ai/kg seed} \times 1\text{kg}/2.2 \text{ lb} \times 1 \text{ lb seeds}/90,000 \text{ or } 115,000 \text{ seeds} \times 1000 \text{ mg/g} = 0.05 \text{ or } 0.04 \text{ mg a.i./seed (depending on seeding rate)}$

$$0.05 \text{ or } 0.04 \text{ mg a.i./seed} \times 150 \text{ seeds/m}^2 \times 4047 \text{ m}^2/\text{A} \times 1 \text{ lb}/454000 \text{ mg} = 0.067 \text{ or } 0.053 \text{ lb a.i./A}$$

2. $7.5 \text{ kg/ha for thiram-treated seeds and } 7.7 \text{ kg/ha for Elado+thiram-treated seeds} = 0.2 \text{ kg Elado/ha} \times 0.333 \times 2.2 \text{ lb/kg} \times 0.405\text{ha}/\text{A} = 0.06 \text{ lb a.i./A}$

Rationale for Use: The results from this study may be used qualitatively in a risk assessment for clothianidin, and provide information on non-*Apis* species which can be used to evaluate potential differences in sensitivity across species. The following limitations are reported.

Comments/Limitations of Study:

1. Characteristics of the soil were not provided (e.g., soil type).
2. The control fields contained just the fungicide while the treated fields contained both a neonicotinoid and a pyrethroid, While the reviewer assumes that direct effects from the pyrethroid are likely low (no detected residues in nectar/pollen and it is not a systemic product), there is uncertainty in discerning the relative impact of the pyrethroid on other environmental factors (For example, indirect effects of beta-cyfluthrin on crop growth and flower density may have impacted the foraging of test bees.)
3. The study authors' state that compared to the wild bees, honey bees were not affected. However, the response variables measured between the wild bees and honey bees are not entirely comparable as weight and/or reproductive parameters were measured in the wild bees, whereas numbers of adults were measured in the honey bees studies as an indirect measurement of fitness. This introduces uncertainty in the ability to compare responses between the species.
4. Raw data are not available.
5. It is unclear if the *O. bicornis* nesting tubes were collected and moved prior to testing their nesting activity in October 2013.
6. It is unclear what the exposure period was for the honey bee hives since the dates in the text and Extended Data Table 2 were not consistent.
7. A description of the site where the honey bee hives were relocated after the oilseed rape flowering period concluded was not provided.
8. Exposure through pollen to *O. bicornis* cannot be confirmed since none were found nesting in the treated fields (therefore no pollen to collect from provisions).
9. Potential bias was introduced in the vegetation sampling and wild and bumblebee monitoring since the observations were not collected blindly.

Description of Use in Document (QUAL, QUAN, INV): Qualitative

Primary Reviewer: Amy Blankinship, EPA/EFED/ERB6

Secondary EPA Reviewer: Michael Wagman, EPA/EFED/ERB6

**MICHAEL
WAGMAN**

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MICHAEL WAGMAN
Date: 2020.01.07 21:44:36
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Secondary PMRA Reviewer: Nicole McKenzie, EAD, PMRA, 9 May 2016

I agree with the primary reviewer's comments and assessment on this study as qualitative.

References: none